Geochemical Perspectives Letters

Magnetotactic bacteria as a new model for P sequestration in the ferruginous Lake Pavin

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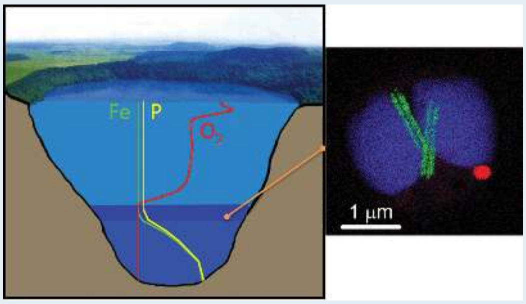
Abstract

doi: 10.7185/geochemlet.1743



The role of microorganisms in the geochemical cycle of P has received great interest in the context of enhanced biological phosphorus removal and phosphorite formation. Here, we combine scanning and transmission electron microscopies, confocal laser scanning microscopy and synchrotron-based x-ray microfluorescence to analyse the distribution of P at the oxic-anoxic interface in the water column of the ferruginous Lake Pavin. We show that magnetotactic bacteria of the *Magnetococcaceae* family strongly accumulate polyphosphates and appear as P hotspots in the particulate fraction at this depth. This high accumulation may be characteristic of this family and may also relate to the chemical conditions prevailing in the lake. As a result, these magnetotactic cocci can be considered as new models playing a potentially important role in the P geochemical cycle, similar to sulphide oxidising bacteria such as *Thiomargarita* and *Beggiatoa* but thriving in a ferruginous, poorly sulphidic environment.

Received 2 June 2017 | Accepted 10 October 2017 | Published 15 November 2017

Introduction

Magnetotactic bacteria (MTB) are a phylogenetically and metabolically diverse group of bacteria biomineralising intracellular magnetites and/or greigites and moving along magnetic field lines. Since their discovery (Blakemore, 1975), they have been found in various environments worldwide including sediments and the water column of freshwater, marine and hypersaline habitats (Lefèvre and Bazylinski, 2013). Their abundance usually peaks at the oxic/anoxic boundary and they have been shown to be the dominant bacteria in some environments (*e.g.*, Spring *et al.*, 1993; Simmons *et al.*, 2007). They have received much attention in the context of the search for ancient traces of life (*e.g.*, Li *et al.*, 2013). They sometimes contribute significantly to the sediment magnetic signal (Chen *et al.*, 2014). However, their effective impact on geochemical cycles has been rarely assessed (Lin *et al.*, 2014) except in a study by Chen *et al.* (2014) suggesting that MTB-associated Fe may provide a significant iron flux in some euxinic systems.

Lake Pavin is a permanently stratified (meromictic) crater lake with a maximum depth of 92 m. The oxic/anoxic boundary is located within the water column, shifting in depth between ~50 to ~65 m depending on the efficiency of water mixing (Michard *et al.*, 1994). In contrast with many permanently stratified water bodies which are euxinic below their chemocline, the monimolimnion of Lake Pavin is ferruginous, *i.e.* sulphide-poor (<20 µM; Bura-Nakić *et al.*, 2009) and Fe(II)-rich (up to 1200 µM; Busigny *et al.*, 2016). The P and Fe cycles are tightly coupled in the anoxic zone through precipitation of Fe phosphates, which impacts the concentration of dissolved P in the deep anoxic waters (Cosmidis *et al.*, 2014; Fig. S-1).

Strong chemical gradients in the water column of Lake Pavin (Fig. S-1) parallel a broad diversity of mineral phases associated with microorganisms, including MTB (Miot *et al.*, 2016). Because the oxic–anoxic interface occurs within the water column of Lake Pavin, it is easily accessible compared to many other aqueous environments, where it is located within the sediments. We therefore studied MTB at the oxic-anoxic interface in Lake Pavin and particularly focused on their association with phosphorus.

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Results

MTB cells were detected by optical microscopy in non-sorted and magnetically-sorted samples collected between 53 and 59 m depth. All MTB-containing samples were collected at a depth where O_2 concentration was lower than the detection limit of the probe (Fig. S-1). Over the sampled depth range, total dissolved Fe, DIP and H_2S concentrations increased from 0.2 to ~60 µM, 0.3 to ~20 µM and 0 to ~2.5 µM, respectively. MTB populations reached a maximum concentration of 1.4 x 10^3 cells/mL. This can be compared to the total number of bacterial cells ranging between 2 x 10^6 and 9 x 10^6 cells/mL in the oxic-anoxic transition zone of Lake Pavin as estimated by Lehours *et al.* (2005). In the MTB population, small curved rods and large rods were observed, but the majority of MTB cells collected at the oxycline were cocci measuring ~2 µm in diameter. Several morphotypes of MTB cocci were observed: the most common contained 1) two chains of cuboctahedral magnetosomes, while some others contained 2) four magnetosome chains or 3) magnetite crystals not aligned as chains (Fig. 1). Many of these MTB cells contained two other types of electron dense granules: S-rich and P-rich granules. S-granules were not systematically observed, although granules that contained only S are shown by EDXS and measured up to ~820 nm in diameter (Fig. S-2). In many MTB cocci, P-rich inclusions filled most of the volume of the cells (Figs. 1, S-2). P-granules contained Ca, K, Mg and P with varying relative abundances. Some MTB cells contained Mg-rich P-granules with relative abundances of Mg, P, K and Ca of 22.8 ± 4, 65.9 ± 3.2, 5.1 ± 3.1 and 6 ± 5.4 at. % (n = 37), respectively. Other MTB cells contained P-granules poorer in Mg and richer in Ca with relative abundances of Mg, P, K and Ca of 6.7 ± 2.5, 56 ± 3.4, 1.5 ± 0.7 and 35.3 ± 1.9 at. % (n = 11), respectively. Similar P-granules have been classically interpreted as polyphosphates (polyP), which are linear polymers of orthophosphate linked by high energy phosphoanhydride bonds (Kornberg, 1995). The (K + Mg + Ca)/P ratio (<0.8) is suggestive of polyphosphates. Here, this interpretation was confirmed by DAPI staining (Figs. 2, S-3).

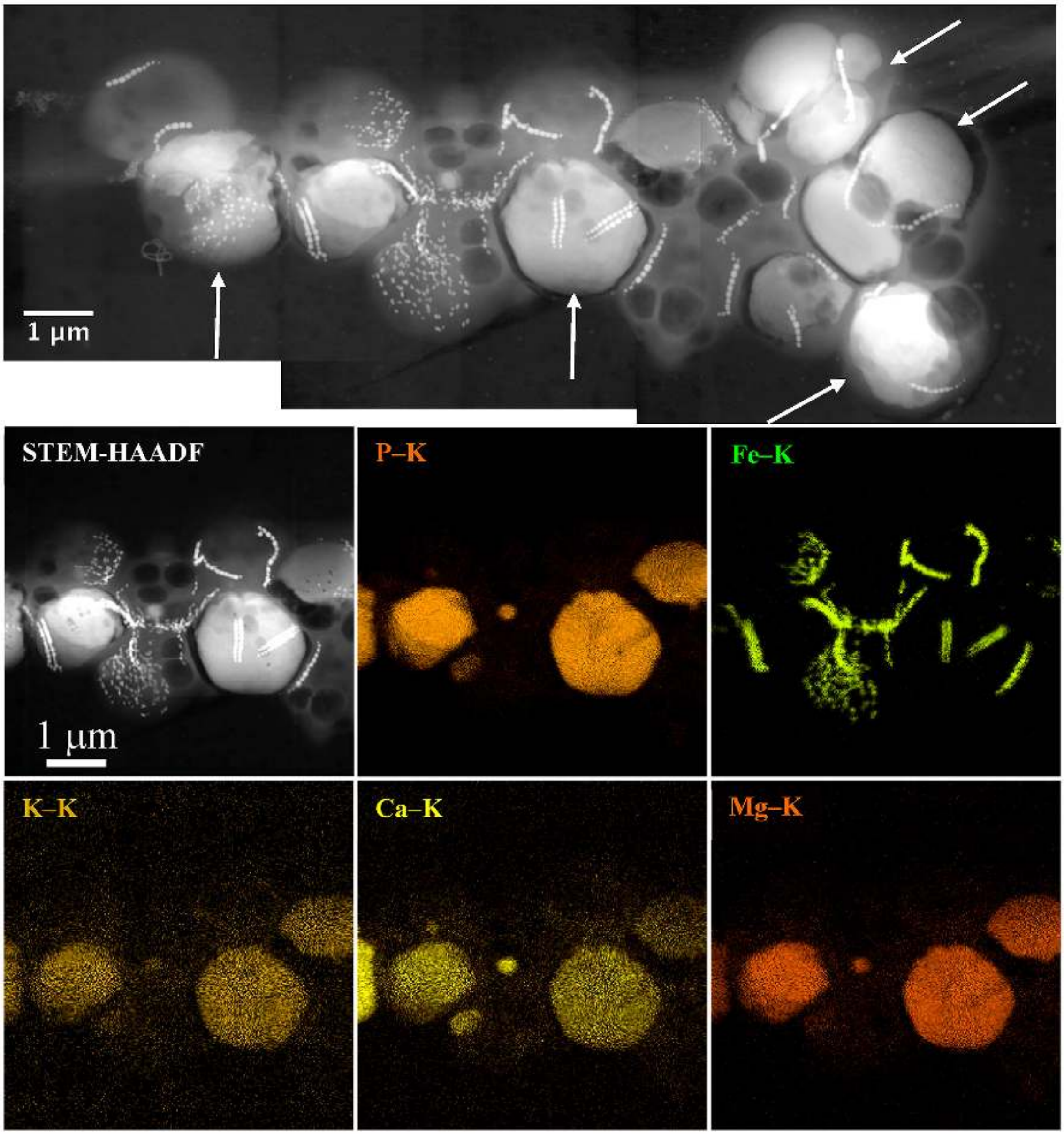

Figure 1 Scanning transmission electron microscopy analyses of MTB cells at the oxic-anoxic interface in Lake Pavin. **Top**: STEM-HAADF image showing a cluster of MTB cells. Magnetites are the brightest particles. Some cells contain two or four magnetite chains; some (bottom left) contain scattered magnetite. Polyphosphates appear as bright granules filling completely some of the cells (*e.g.*, arrows). **Bottom**: EDXS maps.

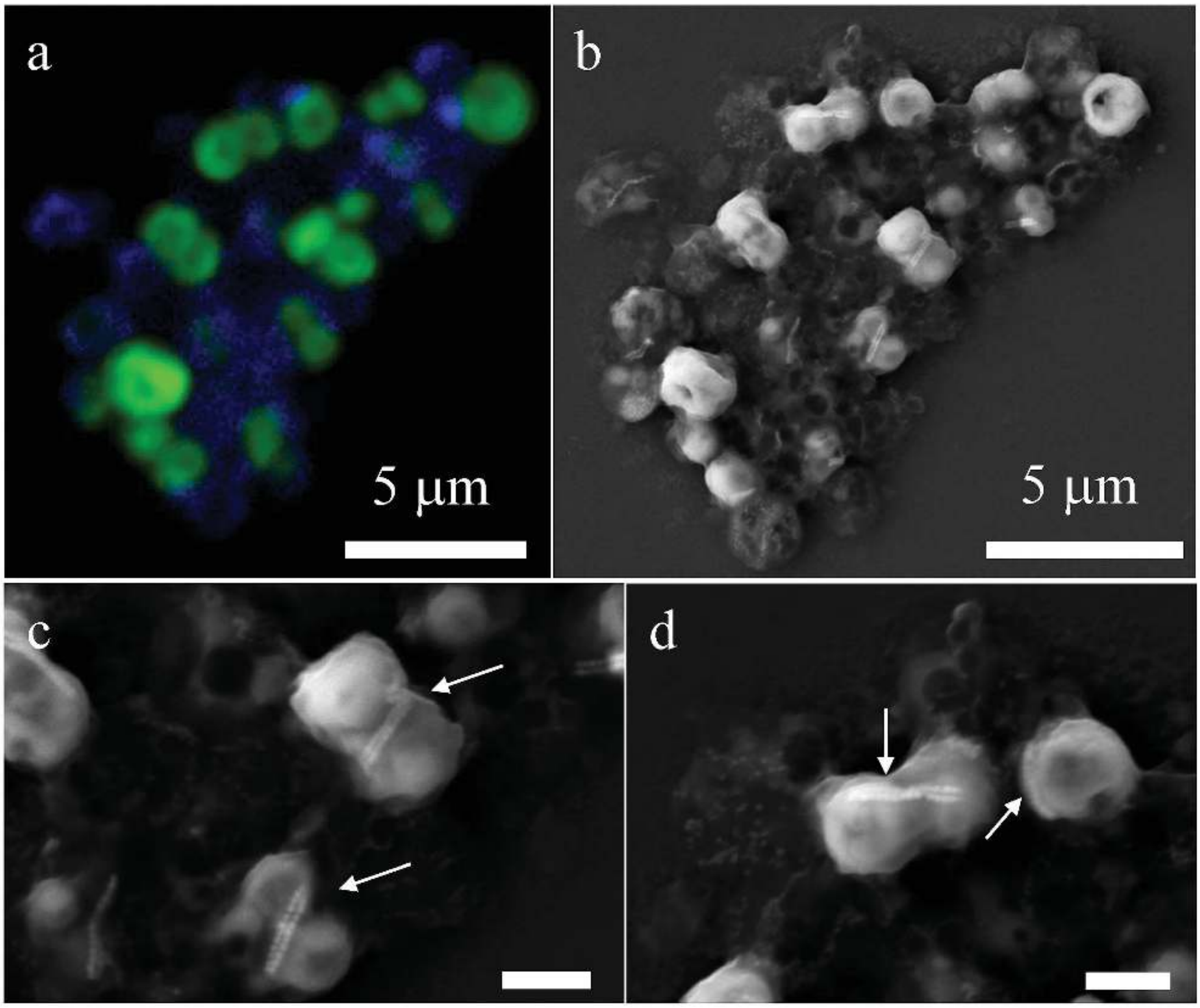

Figure 2 Correlative CLSM-SEM microscopy. **(a)** Overlay of fluorescence maps of DAPI-stained nucleic acid (blue) and polyphosphate (green). **(b)** SEM image in secondary electron mode of the same area. Brighter cells are filled with polyphosphates. **(c)** and **(d)** Close ups of areas seen in (b). Cells filled with polyphosphates contain bright magnetite chains (arrows). Scale bar is 1 µm for (c) and (d).

Phylogenetic analyses revealed that the dominant operational taxonomic units (OTU) from magnetically enriched samples were affiliated to Alphaproteobacteria and more specifically, the *Magnetococcaceae* family (Fig. 3). Based on a similarity threshold of 99 % on the whole 16S rDNA gene sequence for defining species, two different species of magnetotactic cocci were detected. One species (8 clones, accession numbers KX270016-KX270023), represented the most abundant MTB at the oxycline of Lake Pavin. The closest relatives were two magnetotactic cocci (GQ468510 and GQ468517) previously detected in the sediments of Lake Miyun (Beijing, China; Lin *et al.*, 2010). A second species found in smaller proportions (accession number KX270015) was closely related to OTU 13, another magnetotactic coccus detected in Lake Miyun (GQ468512).

In order to better assess the significance of the phosphorus fraction carried by these MTB cells, total particulate matter (>0.2 µm) was collected at the Lake Pavin oxic-anoxic interface and analysed by synchrotron-based x-ray microfluorescence (Fig. 4). The integrated fluorescence intensity at each pixel of the P map was linearly related to the amount of P. We identified 41 areas with high P contents (Fig. S-4). Some areas consisted of a single pixel, whereas others comprised as many as 40 contiguous pixels. All these areas were systematically re-analysed correlatively at higher spatial resolution by SEM. Eight of these P hotspots corresponded to polyP-loaded MTB cells (Fig. 4b,c,e,g,i-l; Fig. S-4). Four other less intense P hotspots were also polyP-loaded MTB cells (Fig. 4a,d,f,h). All the other P hotspots were Fe phosphate precipitates and no other microorganism was detected among these P hotspots (Fig. S-5). Altogether, the 12 polyP-loaded MTB cells identified by SEM harboured 0.6 % of the total P in the mapped area, which comprises a total of 21,336 pixels. Moreover, the P content measured on single MTB-containing pixels was between 6- and 23-fold higher than the median *per* pixel P content (Fig. S-6), providing a rough assessment of the P concentration factor in MTB compared to other cells.

Discussion

Here, we show that some MTB accumulate polyP to a very high level, up to ~23-fold higher than most microbial cells found at the oxic-anoxic interface in the water column of Lake Pavin. High accumulation of polyP was previously documented for several, non-MTB, bacterial species belonging to Proteobacteria and Actinobacteria (*e.g.*, Nakamura *et al.*, 1995; He and McMahon, 2011). Such bacteria have been considered as critical actors for the removal of excess phosphorus from wastewater (Tarayre *et al.*, 2016) or the formation of marine P-rich sediments called phosphorites (Crosby and Bailey, 2012).

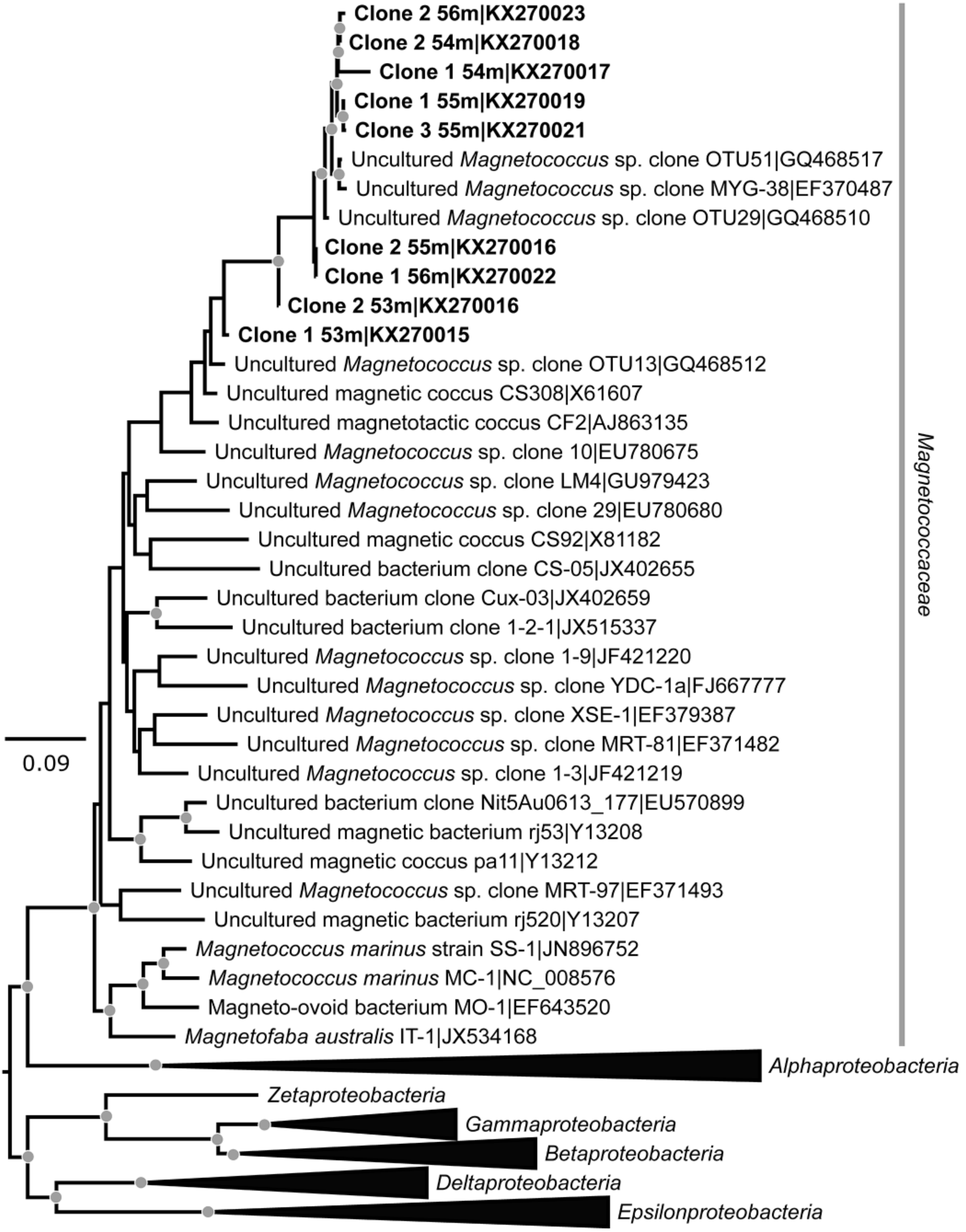

Figure 3 Maximum likelihood phylogenetic tree built based on 16S rDNA gene sequences of MTB clones from Lake Pavin (bold). It includes the closest related *Magnetococcaceae* found elsewhere. GenBank accession numbers are associated with clones/strains names. Nodes supported by a bootstrap value above 70 % are highlighted by a grey circle. Branch length is proportional to the number of base substitutions *per* site (see scale bar).

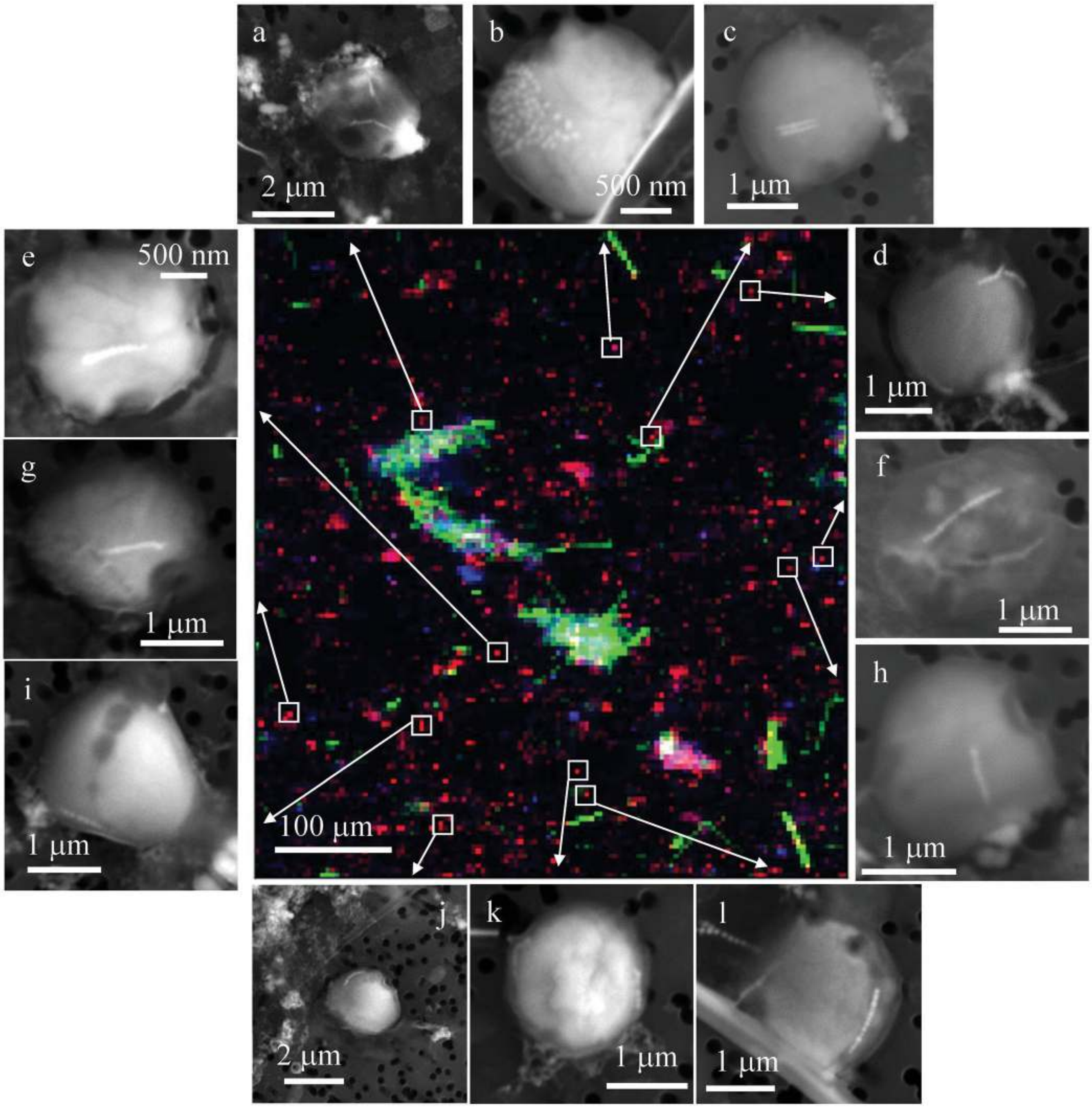

Figure 4 Correlative µ-XRF and SEM microscopy. **Centre**: XRF maps of P (red), Si (green) and S (blue). The brightest P spots were subsequently imaged by SEM. Many hotspots correspond to polyP-loaded MTB cells as shown in **(a)** to **(l)**. Other hotspots were almost exclusively Fe phosphate precipitates (Fig. S-5).

Some magnetotactic cocci from Lake Baldwin, California and Itaipu lagoon, Brazil resembling the MTB cells observed in Lake Pavin were also shown to form intracellular polyP inclusions, sometimes occupying a large part of their cell volume (Cox *et al.*, 2005; Keim *et al.*, 2005). This process is even more pronounced in Lake Pavin with some *Magnetococcaceae* cells fully filled with polyP. Here, the ability of these cells to swim along the magnetic field lines during magnetic enrichment procedures suggests that they stay viable.

Several functions have been suggested for polyP inclusions such as a source of ATP, or a response to oxidative stress (Seviour *et al.*, 2003). MTB cells containing large amounts of polyP in Lake Pavin were detected at depths where low extracellular P concentrations (~0.7 µM at 54 m) were measured. Several environmental conditions have been shown to stimulate polyP accumulation/hydrolysis (*e.g.*, Brock and Schulz-Vogt, 2011). Firstly, in wastewater treatments, addition of fatty acids or acetate under anoxic conditions triggers phosphate release by some polyP-concentrating bacteria (Comeau *et al.*, 1986), while these bacteria accumulate polyP under oxic conditions (*e.g.*, Karl *et al.*, 2014). Oxic-anoxic cycles induce high polyP accumulation in these bacteria. Lake Pavin *Magnetococcaceae* may massively accumulate polyP by experiencing similar oxic/anoxic fluctuations, either by travelling vertically over short distances from the oxic to anoxic zone (and *vice versa*), or by being affected by seasonal depth shifts of the oxic/anoxic boundary. Accumulation and hydrolysis of polyP may affect cell buoyancy as shown for other bacteria (Romans *et al.*, 1994) and favour downward movements after accumulation. Secondly, and alternatively, addition of sulphide under anoxic conditions triggers phosphate release by the sulphoxidising bacteria *Beggiatoa* and *Thiomargarita* (Brock and Schulz-Vogt, 2011), while these bacteria accumulate and keep high polyP contents under oxic conditions and anoxic sulphide-poor conditions. Interestingly, intracellular S-globules in some of Lake Pavin MTB cells suggest that these bacteria are sulphide and/or thiosulphate oxidisers, similar to the closely genetically related cultured *Magnetococcus marinus* strain MC-1 (Bazylinski *et al.*, 2013). Whether Lake Pavin MTB are affected by sulphides similarly to *Beggiatoa* will have to be determined. If this is the case, sulphide concentrations are relatively low (<0.2 µM below 58 m) at the depth where Lake Pavin MTB were observed and could be a parameter explaining the high accumulation of polyP in MTB below the oxic/anoxic boundary in Lake Pavin. Analysing the polyP content of MTB cells at different depths throughout these chemical gradients would help in answering this question.

Fe phases were suggested to control the P geochemical cycle in Lake Pavin (Busigny *et al.*, 2016) *via* high affinity sorption at their surface. Alternatively, involvement of microorganisms in the extracellular precipitation of Fe phosphates has been evidenced (Cosmidis *et al.*, 2014; Miot *et al.*, 2016). Here, MTB also couple the geochemical cycles of P and Fe through accumulation of two separate Fe and P intracellular reservoirs (polyP and magnetites).

The geochemical impact of polyP-accumulating bacteria has been particularly stressed in the context of wastewater treatment and the formation of phosphorites. In the first case, bacterial genera such as *Pseudomonas* and *Acinetobacter* play a predominant role in P accumulation in P-rich solutions (Nathan *et al.*, 1993). In the case of marine phosphorite formation, sulphoxidising bacteria have become major bacterial models involved in the accumulation of low concentrations of P from the water column, before release in porewater under anoxic conditions, resulting in the precipitation of apatite-like phases in the sediments (Goldhammer *et al.*, 2010). Microfossils resembling modern sulphoxidising bacteria were evidenced in the geological record, suggesting that they may have played a role in the formation of ancient phosphorites as well (Bailey *et al.*, 2013). Diatoms have been also suspected to be major players in the accumulation of P as polyP, before release and precipitation of P phases in the sediments (Diaz *et al.*, 2008). Here, we show that *Magnetococcaceae* appear as new models of polyP-accumulating bacteria in freshwater ferruginous environments. While the reservoir they represent seems modest in size (<1 % of the total P), it may, similarly to sulphoxidising bacteria in some marine sediments, be dynamic because of active microbially mediated transformations of polyP and may therefore significantly contribute to the P cycle in this environment. This will be important to assess further in future studies. The relative simplicity by which MTB can be extracted from a suspension by magnetic sorting makes them an interesting tool for concentrating and removing P from a solution. Moreover, similar to sulphide oxidising bacteria, traces of MTB are tractable in the geological record. Identifying the specific crystallographic, geochemical and/or magnetic properties (*e.g.*, Amor *et al.*, 2016) of their intracellular magnetites provides a perspective to track their potential contribution to the formation of ancient lacustrine phosphorites.

Acknowledgements

KB has been supported by funding from the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013 Grant Agreement no.307110 – ERC CALCYAN). The TEM and SEM facilities at IMPMC were purchased owing to a support by Region Ile-de-France grant SESAME 2000E1435 and 2006N°I-07-593/R. CM and CL were supported by the French National Research Agency (ANR Tremplin-ERC BIOMAGNET and ANR MEFISTO). ED was supported by the French National Center for Scientific Research (CNRS-INSU program EC2CO). JM was supported by the ANR SRB project, grant ANR-14-CE33-0003-01 of the French Agence Nationale de la Recherche.

Editor: Liane G. Benning

Additional Information

Supplementary Information accompanies this letter at www.geochemicalperspectivesletters.org/article1743

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Cite this letter as: Rivas-Lamelo, S., Benzerara, K., Lefèvre, C.T., Monteil, C.L., Jézéquel, D., Menguy, N., Viollier, E., Guyot, F., Férard, C., Poinsot, M., Skouri-Panet, F., Trcera, N., Miot, J., Duprat, E. (2017) Magnetotactic bacteria as a new model for P sequestration in the ferruginous Lake Pavin. *Geochem. Persp. Let.* 5, 35–41.

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